METAL LEVELS IN SEVEN SPECIES OF MOLLUSC AND IN SEAWEEDS FROM THE SHANNON ESTUARY

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ABSTRACT

Metal levels (iron, zinc, manganese, cobalt, chromium, copper and nickel) were determined in *Cerastoderma edule*, *Mytilus edulis*, *Monodonta lineata*, *Patella vulgata*, *Nucella lapillus*, *Littorina obtusata* and *L. littorea* at two sites close to the mouth of the Shannon Estuary (Ireland) in November 1993 and May 1994, using standard atomic absorption spectrophotometer techniques. Samples of seaweeds were included at one shore for comparative purposes only. The best all-round indicators for all the metals studied were *C. edule*, *M. edulis* and *N. lapillus*. Metal levels in the seaweeds were much lower than in the molluscs. No species was a universal indicator of the metals studied when site and season were taken into consideration. High levels of metals accumulated from water and/or sediment were observed in a number of cases. Levels of Zn in *N. lapillus* were significantly different to those in all other species and *C. edule* had high levels of Ni. These levels were dependent on site and season. A detrended correspondence ordination showed that the two species of bivalve mollusc and *P. vulgata* formed one cluster and that the remaining gastropod species formed a second cluster. This study shows that species-specific bioaccumulation occurs and is seasonally specific in certain species. This needs to be taken into account when choosing a bioaccumulator model and when comparing data from different studies.

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INTRODUCTION

Bivalve and gastropod molluscs are frequently used to biomonitor heavy metal pollution in the estuarine environment (e.g. Bryan *et al.* 1985; Phillips 1980; Wilson 1980; 1982). Each class includes bioaccumulating species that may be sedentary, filter-feeding and/or long-lived, and tolerant of the varying salinity levels and pH values found in estuaries (McLusky 1989; Hayward and Ryland 1995). Metals may be bioaccumulated from solution, suspended particles, sediment, seaweed and phytoplankton, and the uptake of metals may differ between species because of the different modes of feeding (Bryan *et al.* 1985). Certain marine molluscs regulate their body tissue levels of particular trace metals to constant levels over a wide range of metal levels in their environment (see Rainbow *et al.* 1990), and laboratory experiments have also provided evidence that this regulation is species-specific (Bryan *et al.* 1985; Rainbow *et al.* 1990; Dallinger and Rainbow 1993).

According to Bryan *et al.* (1980) it is unlikely that any one species would be a universal indicator organism capable of assessing contamination in all available forms, as different species have different affinities for metals and absorption may be from different sources. A rationale behind the present study is that the analysis of metal levels in a range of different species, combined with a consideration of their comparative biology, may suggest the source of a particular metal in their environment. Rainbow *et al.* (1990) pointed out that gastropod molluscs are used much less extensively as potential biomonitors in aquatic ecosystems than bivalve molluscs. Seaweeds, especially Phaeophyta, have frequently been used as biomonitors of metal levels. Both gastropods and seaweeds were included in the present study for comparative purposes.

Although metal levels in *Mytilus edulis* and *Ostrea edulis* from the Shannon Estuary were included in once-off studies of Irish estuaries (Nixon *et al.* 1991; O'Sullivan *et al.* 1991), the first long-term analysis of metal levels in *M. edulis* in this estuary was carried out by O'Leary (1995), based on 22 monthly samples at five sites during 1992–3. An overall conclusion of this study was that metal levels were low, both in comparison to other Irish sites and internationally. There are no previous studies of metal levels in *Cerastoderma edule*, *Monodonta lineata*, *Patella vulgata*, *Nucella lapillus*, *Littorina obtusata* (formerly *L. littoralis* and separated from *L. mariae* on the basis of shell morphology; see Hayward and Ryland 1995), *L. littorea* and seaweeds in the Shannon Estuary.

Consequently, the aims of this paper are (i) to provide a comparative study of the variation in metal levels between a number of sublittoral molluscs and seaweeds from the Shannon Estuary, and (ii) to investigate differences between species, sites and seasons and their implications for the use of these organisms for the biomonitoring of metal levels.

METHODS

SITE

The Shannon Estuary, located in mid-western Ireland, is the largest estuary in the country and is approximately 100km long. For further details, including a map, see O'Leary 1995. The two study sites were Bunaclugga Bay, south coast (Irish Grid Q95 47), and Querrin Point, north coast (Irish Grid Q93 53), two sites located on opposite shores at the mouth of the estuary. Both sites are fully saline (McMahon 1988; Curran 1992) but are within the lower limits of the Shannon Estuary (O'Gara *et al*. 1990). Samples were collected at Bunaclugga Bay in November 1993 and May 1994 (referred to as shores 1w and 1s, for winter and summer respectively) and at Querrin Point in May 1994 (referred to as shore 2s, for summer).

SAMPLING

Samples (fifteen individuals of each species) of *Cerastoderma edule*, *Mytilus edulis*, *Monodonta lineata*, *Patella vulgata*, *Nucella lapillus*, *Littorina obtusata* and *L. littorea* were collected from shores 1w, 1s and 2s, except that *M. lineata* was not present at shore 2s. A range of sizes was taken to provide a cross-section of size and probably of both mature and immature individuals. Samples were not sexed. Samples (taken from fifteen different plants) of *Ulva lactuca*, *Chondrus crispus*, *Pelvetia canaliculata*, *Fucus serratus* (current year's growth) and *F. vesiculosus* (current year's growth) were collected at shore 1s.

SAMPLE PREPARATION

Molluscs were allowed to depurate for five days. The preparatory techniques broadly follow the procedures of Bryan *et al*. (1980) and Söderlund *et al*. (1988) and are detailed in O'Leary 1995. After removal from the freezer (−18°C), mollusc samples were allowed to defrost overnight, and then allowed to drain for five minutes. Soft body tissues were removed from shells, dried, weighed and digested individually in concentrated nitric acid (Greenberg *et al*. 1989). Tissue levels of Co, Cr, Cu, Fe, Mn, Ni and Zn were analysed with a Varian atomic absorption spectrophotometer, using IAEA reference material as a control. The optimum working range values were Co 3–12μg ml^{-1}, Cr 2–8μg ml^{-1}, Cu 2–8μg ml^{-1}, Fe 2.5–10.0μg ml^{-1}, Mn 1–4μg ml^{-1}, Ni 3–12μg ml^{-1} and Zn 0.4–1.6μg ml^{-1}. Values below the detection limits of the atomic absorption spectrophotometer are reported as zero values.

Seaweed samples were washed three times, in sea water, in distilled water and in sea water again. Epifauna and epiphytes were removed. Samples were then scrubbed in sea water using a nylon brush, rinsed in distilled water and finally in sea water. After air-drying at room temperature for three to four days, they were stored in polythene bags until analysis. The digestion and analysis procedures were the same as for the molluscs.

DATA ANALYSIS

After testing for normality, all data sets were transformed to natural logarithms (of $x+1$, to avoid errors when values of zero occurred) prior to statistical analyses, using analysis of variance (ANOVA) with Duncan's *a posteriori* tests, and Pearson correlation coefficient using SPSS for Windows (Norušis 1993). Finally, a detrended correspondence analysis (DCA) ordination was obtained using MVSP (Kovach 1991). DCA provides two-dimensional plots of multidimensional ecological and species data (see Digby and Kempton 1987).

RESULTS

Mean values and the standard errors of the metal levels in seven species of mollusc from shores 1w and 1s (November 1993 and May 1994) and shore 2s (May 1994) are plotted in Fig. 1. The levels were all low, with the exception of Fe. Levels of Fe in *Cerastoderma edule*, *Mytilus edulis* and *Patella vulgata* at shores 1s and 2s were high in comparison with shore 1w and with the other results obtained by O'Leary (1995) based on five shores over 22 months (1993–4) in *M. edulis*. In *Littorina littorea* the levels of Ni were high in winter (1w) but low in summer (1s and 2s), whereas levels of Co were higher in summer (1s) compared to the previous winter levels (1w). Ni levels in *C. edule* were also high on both shores in summer (1s and 2s). Cu levels were highest in *Nucella lapillus*; similar Cu levels occurred in *Monodonta lineata* but only at shores 1w and 1s. The highest levels of Mn were in *C. edule* at shore 1w but this level was not repeated. Zn was highest in *N. lapillus* at shores 1w and 2s, whereas the level at shore 1s was considerably lower.

A one-way ANOVA for each metal and species at Bunaclugga Bay (shore 1w) in November 1993 (Table 1) is read as follows: levels of Co in *C. edule* [a] were significantly different to those in *N. lapillus* [d] and in *P. vulgata*, *M. lineata*, *L. Littorea*

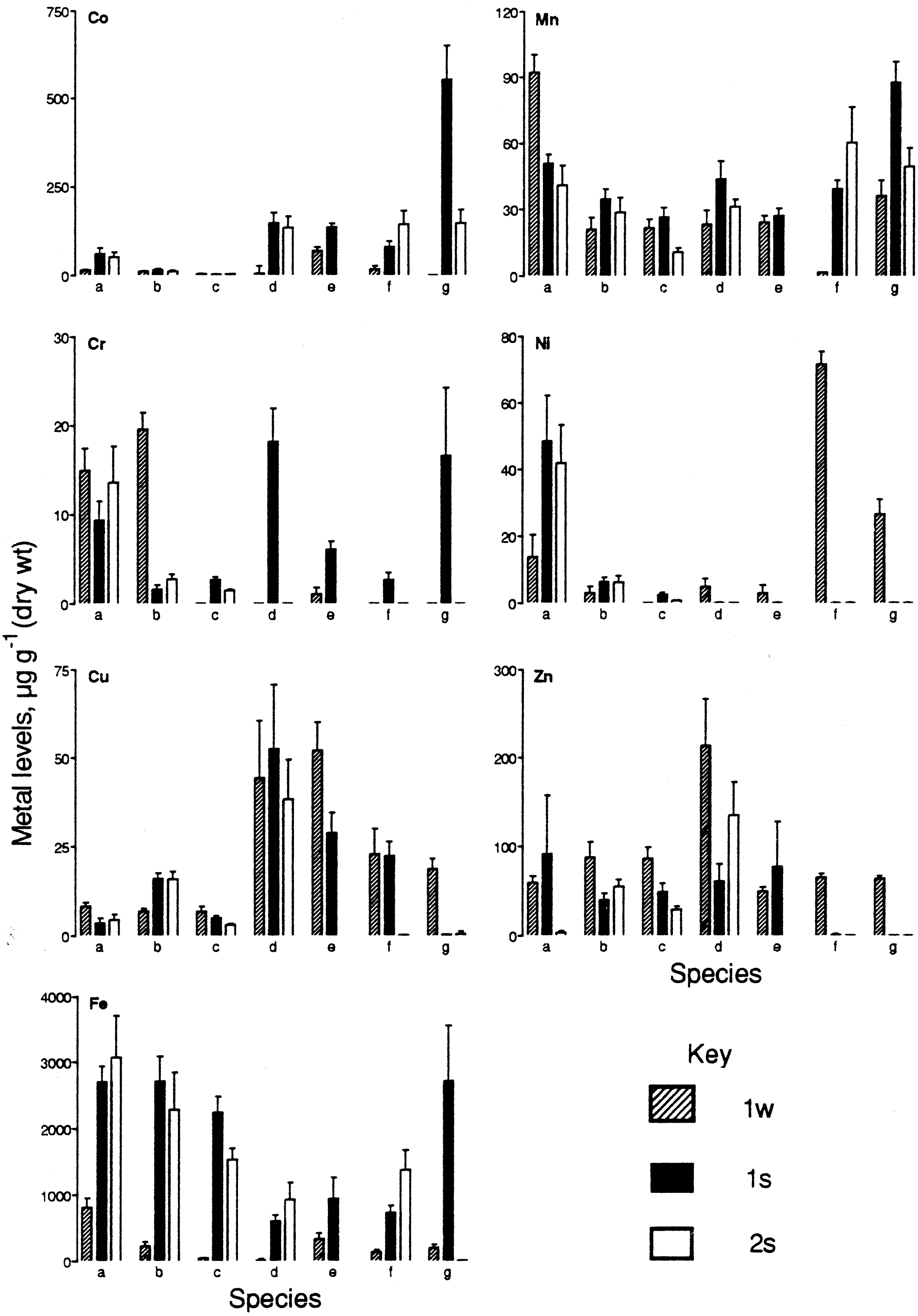

Fig. 1—Mean values (±S.E.) of Co, Cr, Cu, Fe, Mn, Ni and Zn levels in seven species of mollusc from shores 1w (November 1993, cross-hatched pattern), 1s (May 1994, black) and 2s (May 1994, white). Key to species: a = *Cerastoderma edule*, b = *Mytilus edulis*, c = *Patella vulgata*, d = *Nucella lapillus*, e = *Monodonta lineata*, f = *Littorina littorea*, g = *Littorina obtusata*.

and *L. obtusata* [c, e, f, g], each of which had Co levels which were not significantly different from each other. Some interesting points may be noted: levels of Zn in *N. lapillus* were significantly different to all other species analysed; levels of Cr in *C. edule* and *M. edulis* were significantly different to all other species; levels of Ni in *L. obtusata* and *L. littorea* were significantly different to *P. vulgata*, *M. lineata*, *M. edulis*, *N. lapillus* and *C. edule*; Mn levels in *C. edule* and *L. littorea* were significantly different to all other species studied; and levels of Cu in *M. edulis* were significantly different to *L. obtusata*, *L. littorea*, *N. lapillus* and *M. lineata*.

The different magnitudes of scale for each metal are a problem when comparing the bioaccumulation of the different metals. In particular, the levels of Fe are very high and this precludes graphing with levels of the other metals together. Consequently, the following approach was devised to graph all species and metals together: metal levels in individual specimens were expressed as a percentage of the highest value obtained for each

Table 1—Means ($\bar{x}$) and standard errors (S.E.) of Co, Cr, Cu, Fe, Mn, Ni and Zn in species a–g (defined in key to species) at shore 1w and compared using Duncan's *a posteriori* test, where groups of species not significantly different from each other at $P = 0.05$ are enclosed in brackets.

Species	*Metal*	$\bar{x}$	*S.E.*	*Duncan's*	*Metal*	$\bar{x}$	*S.E.*	*Duncan's*
	Co				Mn			
a		16.13	1.31	[d][c e g f]		2.04	8.24	[b c d e f g]
b		12.50	1.11	[d][c e g f]		21.15	5.25	[a][f]
c		5.78	0.70	[e f g][a b d]		21.72	3.84	[a][f]
d		70.39	10.34	[a b c e f g]		23.42	6.24	[a][f]
e		0.00	0.00	[a b c d f]		24.46	2.93	[a][f]
f		18.17	8.69	[e f][a b c d]		2.00	0.00	[a b c d e g]
g		0.00	0.00	[a b c d f]		36.60	6.76	[f][a]
	Cr				Ni			
a		14.96	2.48	[c d e f g][d]		13.89	6.57	[c]
b		19.59	1.85	[a c d e f g]		3.04	1.94	[f g]
c		0.00	0.00	[a b]		0.03	0.03	[a f g]
d		0.00	0.00	[a b]		5.11	2.35	[f g]
e		1.10	0.75	[a b]		3.03	2.52	[f g]
f		0.00	0.00	[a b]		71.92	3.86	[a b c d e g]
g		0.00	0.00	[a b]		26.67	4.54	[a b c d e]
	Cu				Zn			
a		8.37	0.95	[d e g]		60.05	7.28	[d]
b		6.99	0.70	[d e f g]		87.86	17.77	[d]
c		5.64	1.29	[d e f g]		87.03	12.56	[d]
d		44.54	15.97	[a b c f g]		213.79	53.14	[a b c e f g]
e		52.23	7.86	[a b c f g]		50.09	5.31	[d]
f		22.93	7.29	[b c][d e]		66.09	4.19	[d]
g		18.89	2.87	[a b c][d e]		64.58	2.88	[d]
	Fe							
a		811.83	134.75	[bc d f g]				
b		234.77	63.62	[a][d]				
c		49.23	6.88	[a e][d]				
d		21.24	21.24	[a b c e f g]				
e		341.36	89.47	[c d f]				
f		148.55	31.48	[a e][d]				
g		211.4	46.32	[a][d]				

Key to species

a = *Cerastoderma edule*
b = *Mytilus edulis*
c = *Patella vulgata*
d = *Nucella lapillus*
e = *Monodonta lineata*
f = *Littorina littorea*
g = *Littorina obtusata*

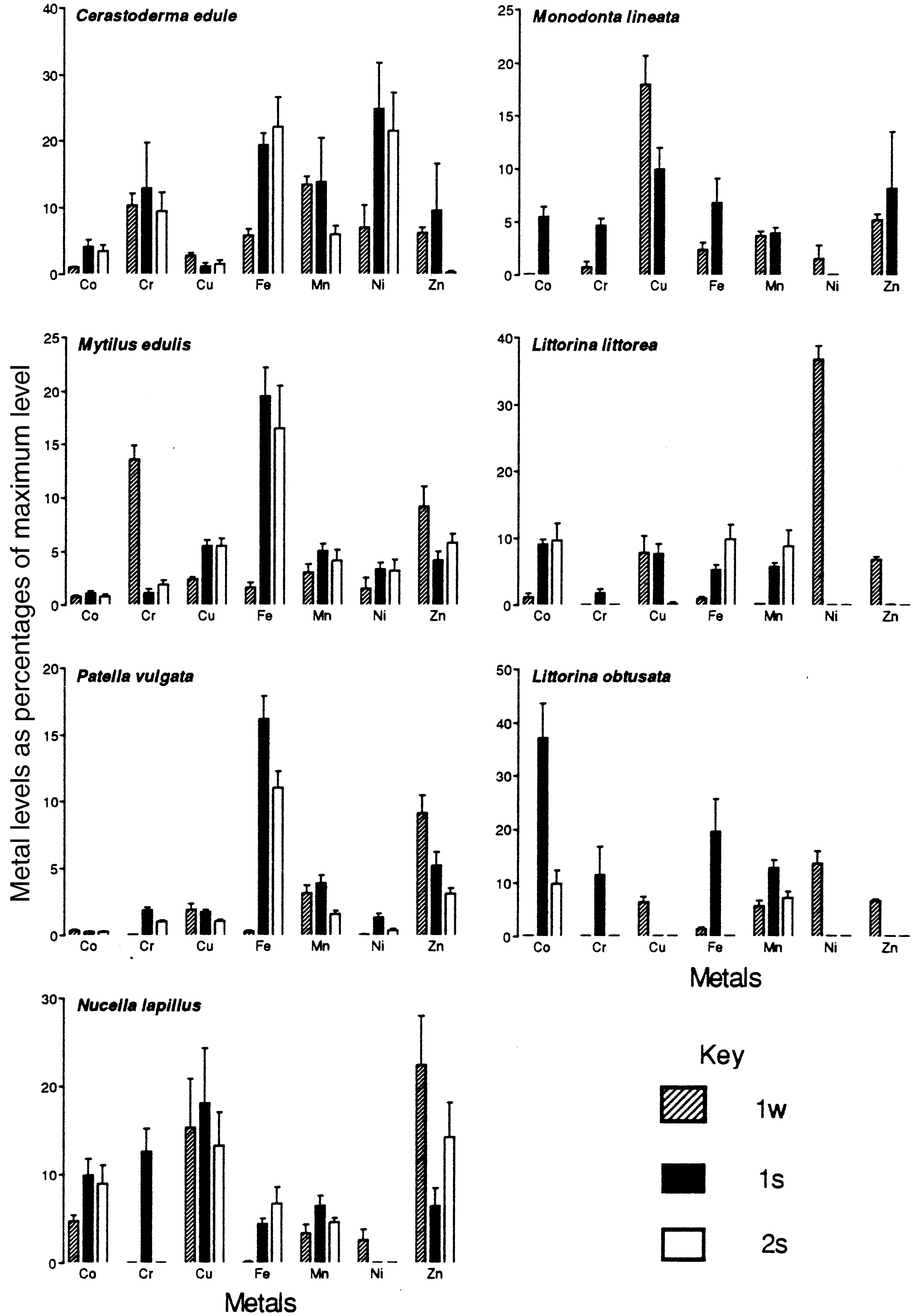

Fig. 2—Mean values (±S.E.) of Co, Cr, Cu, Fe, Mn, Ni and Zn levels expressed as a percentage of the maximum value of each metal (see text) in seven species of mollusc from shores 1w (November 1993, cross-hatched pattern), 1s (May 1994, black) and 2s (May 1994, white).

Table 2—Significant Pearson's correlations between combinations of the metals Co, Cr, Cu, Fe, Mn, Ni and Zn and dry weight on shores 1w, 1s and 2s (see main text); + and − denote the sign of the correlation. One symbol = $P < 0.05$; two symbols = $P < 0.01$; three symbols = $P < 0.001$. Key to species: a = *Cerastoderma edule*, b = *Mytilus edulis*, c = *Patella vulgata*, d = *Nucella lapillus*, f = *Littorina littorea*, g = *Littorina obtusata*.

		Species						
	Shore	*a*	*b*	*c*	*d*	*e*	*f*	*g*
Co–Cr	1w		+++					
	2s	++		+++				
Co–Cu	1w		+					
	1s		+					
	2s		++	+				
Co–Fe	2s	+++		++				
Co–Mn	1w						+++	+++
	1s		+		+++	+		
	2s	+++		+				
Co–Ni	1w							
	1s							
	2s	++						
Co–Zn	1s				−			
	2s							
Co–dry wt	1w	−−	−−−		−−−			
	1s		−−		−−−	−−−	−−−	−
	2s	−−−	−−	−−−				
Co–shell wt	1w			−−−				
	1s				−−−			
Cr–Cu	1s			+++				
	2s			+++				
Cr–Mn	1w	+						
	1s			+				
	2s	++		++				
Cr–Ni	1s			++				
	2s	++						
Cr–Fe	1w	+						
	1s	−		++				
	2s	+		−−−				
Cr–Zn	1s			++				
	2s			+				
Cr–dry wt	1w		−−					
	1s			−−−			−	
	2s	−		−−−				
Cu–Mn	1w			+	+++	+++		
	1s	+	++					++
	2s		+++	+++				
Cu–Fe	1w			+++	++			
	1s			++		+		++
	2s		+++	+++				
Cu–Zn	1w			+	+++	−−		
	2s			+++				

metal. Mean percentage levels ($\pm$S.E.) are plotted in Fig. 2. Since the metals are now all plotted on the same percentage scale, the histograms can be examined for consistency within each species and metal. Species with consistent levels and low standard errors, especially on the two shores sampled in summer (1s and 2s), act as good indicators.

Figure 2 shows that no species was a universal indicator for all seven metals studied, although *C. edule*, *M. edulis* and *N. lapillus* were good indicators of groups of metals. *Cerastoderma edule* was a good overall indicator of Cr, Mn, Ni and Fe at shores 1s and 2s, and of Zn at shores 1w and 1s. It was also a consistent indicator of Co and Cu, although the

Table 2—(continued).

		Species						
	Shore	*a*	*b*	*c*	*d*	*e*	*f*	*g*
Cu–Ni	1s			++				
	2s	+						
Cu–dry wt	1w	−−		−−−				
	1s	−		−−				
	2s			−−				
Cu–shell wt	1w			−−				
	1s			+				
Fe–Mn	1w				++	−		
	1s	+	+	++			++	+++
	2s	+++	+++	+++	+++			
Fe–Ni	1s			++				
	2s	++						
Fe–Zn	1w			+	+++			
	1s		+	++				
	2s			+++				
Fe–dry wt	1w			−−−				
	1s			−−−		−		
	2s	−		−−−				
Fe–shell wt	1w			−−				
	1s							
	2s					+		
Mn–Ni	1s			++				
	2s	+++						
Mn–Zn	1w	++		++		−−		
	1s		++	+	−−−			
	2s			++				
Mn–dry wt	1w	−−						
	1s	−−			−−−	−−−		
	2s	−−		−−			−−	−
Mn–shell wt	1s				−−−			
Ni–Zn	1w			−−			+++	+++
	1s			++				
Ni–dry wt	1s			−				
Zn–dry wt	1w			−				
	1s			−	+			

overall levels of these metals were low (see Fig. 1). *Mytilus edulis* was a good overall indicator of Co, Cu, Mn, Ni and Zn, but extreme variations were obtained for Fe and Cr. *Patella vulgata* was a good indicator of Cu and Mn and *N. lapillus* provided consistent results for Co, Cu and Mn. *Monodonta lineata* (which did not occur on shore 2s) and *L. obtusata* were specific for Mn but provided inconsistent results for the other metals.

Figure 3 shows the metal levels in the seaweeds at shore 1s; levels of Co and Ni were below the detection limits in all seaweed species. *Ulva lactuca* accumulated Fe, Mn, Cu and Ni. *Chrondrus crispus* accumulated high levels of Cu, *Pelvetia canaliculata* accumulated a high level of Zn, and *Fucus vesiculosus* was specific for Zn and Mn.

Pearson correlation coefficients between different combinations of metals (raw values, $\log_e$ transformed), dry weights and shell weights for each species and each shore are given in Table 2. Note the clusters of correlations, particularly those at $P < 0.01$, for the species, and the similarities and differences between seasons and sites. Positive correlations in *P. vulgata* included Fe–Zn and Mn–Zn in both seasons (shores 1w, 1s and 2s) and Cr–Cu, Cr–Zn and Fe–Mn only in summer (shores 1s and 2s). Dry weights were negatively correlated with many combinations of species, site and metal levels but most noticeably in *C. edule* and *M. edulis*. A number of metal levels were negatively correlated with shell weight in *P. vulgata* and *N. lapillus*, though in this case covariance between shell and dry weight is likely.

A DCA of the data from shore 1w is shown in Fig. 4 (axis 1: eigenvalue 0.171, 41.92% of variation; axis 2: eigenvalue 0.079, 19.29% of variation). The plot of the metal levels shows Cr at the low end and Ni at the high end of axis 1, with Mn, Zn, Fe and Cu in a cluster in the middle. On axis 2, Co and Ni are at the high end, Fe at the opposite end and Cr, Mn, Zn and Cu in the middle. The species plot shows an interesting ordination of the species. The bivalves *C. edule* and *M. edulis* cluster together alongside *P. vulgata*. With two exceptions (the *Littorina* species), individuals from the other species cluster tightly on their own. The points for *L. obtusata* and *L. littorea* are relatively widely scattered towards the higher end of both axes 1 and 2. Direct comparison is allowed in DCA between the environmental (chemical) and species plots and it is possible to infer higher or lower levels of individual metals in individual species in this way. Thus, species with high Cr levels (*C. edule*, *M. edulis*) cluster on the left-hand side of the species plot, whereas species with relatively high Ni values (*L. obtusata* and *L. littorea*) appear at the right-hand side of the plot.

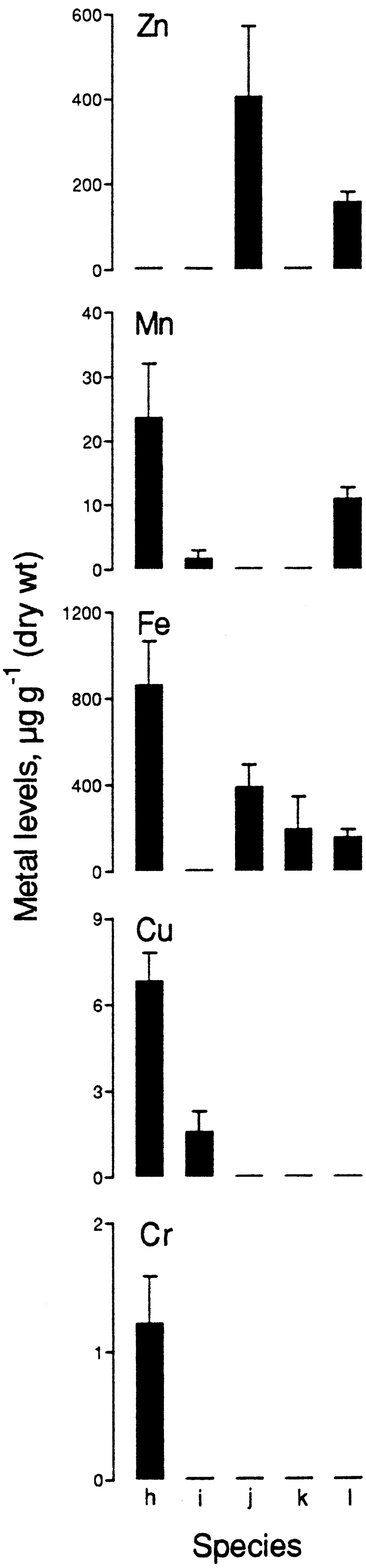

Fig. 3—Mean values (±S.E.) of Co, Cr, Cu, Fe, Mn, Ni and Zn in seaweeds at shore 1s. Key to seaweeds: h = *Ulva lactuca*, i = *Chondrus crispus*, j = *Pelvetia canaliculata*, k = *Fucus serratus*, l = *Fucus vesiculosus*.

DISCUSSION

OVERVIEW

No species was a universal indicator of the metals studied when site and season were taken into consideration. Species-specific metal accumulation was observed in a number of cases and was dependent on site and season. For example, *Monodonta lineata*, a detrital sweeper (Hayward and Ryland 1995), had high levels of Cu at shore 1s (Fig. 1) but showed more constant accumulation of all metals at shore 2s. Some of the Cu levels in marine molluscs may be attributed to the presence of the copper-containing blood pigment haemocyanin (Barrington 1980). *Littorina obtusata* and *L. littorea* had high levels of Ni in winter but not in summer. Comparisons between estuaries are difficult because of the inherent variability in underlying bedrock, sediment type and anthropogenic inputs. However, some comparisons with studies of the east coast of Ireland and Britain are included below where relevant. The metal levels reported here are lower than in these studies, with a few exceptions.

High Fe levels were evident in all the molluscs studied in summer. These were the highest levels of Fe obtained during an extended biomonitoring study in the Shannon Estuary (O'Leary 1995), and may indicate a once-off anthropogenic input or release from bedrock or sediment (Zauke and Petri 1993) by a disturbance such as dredging. However, since the elevated levels were of Fe only, an anthropogenic source seems more likely.

Cerastoderma edule

Many authors (e.g. Bryan *et al.* 1980; Wilson 1980; 1983) have stated that *C. edule* (species a in Fig. 1) is a good indicator of Ni. The results presented here (Fig. 1) support this, as levels of Ni for *C. edule* were higher than in the other species studied. Furthermore, within *C. edule* Ni had the highest percentage accumulation (Fig. 2). Accumulation of Cr and Cu by *C. edule* was consistent for sites and seasons (Figs 1 and 2). This is not in agreement with Bryan *et al.* (1985), who considered *C. edule* a moderate indicator of Cr and a moderate to poor indicator of Cu. The results obtained in this study show that *C. edule* is a poor indicator of Zn, which is in agreement with Bryan *et al.* (1985).

The levels of Fe, Zn, Cu and Mn in *C. edule* reported by Bryan and Gibbs (1983) from the Fal Estuary, south-west England, were higher than those from the Shannon Estuary. These authors suggested that their high Fe levels were due to the *C. edule* being incompletely purged of sediment particles because they were moribund prior to analysis. Since the *C. edule* collected from the Shannon Estuary were alive on analysis and were allowed to depurate for three days, the high Fe levels found were not due to an error in analysis but to body tissue levels accumulated by the organism. Furthermore, the levels of the other metals, including Cu and Zn, were within normal limits. Thus it appears that the levels of Fe reported here from *C. edule* are due to tissue levels rather than to sediment in the intestine. In another study (O'Leary 1995) the Fe levels reported from Shannon Estuary sediments were also high. It is possible that Fe in *C. edule* comes from the sediment as *C. edule* is a suspension-feeding bivalve, living in the sediment.

Wilson (1980) reported levels of various metals in *C. edule* from a number of east-coast sites in winter. In the Shannon Estuary, winter levels of Fe were considerably lower, and summer levels

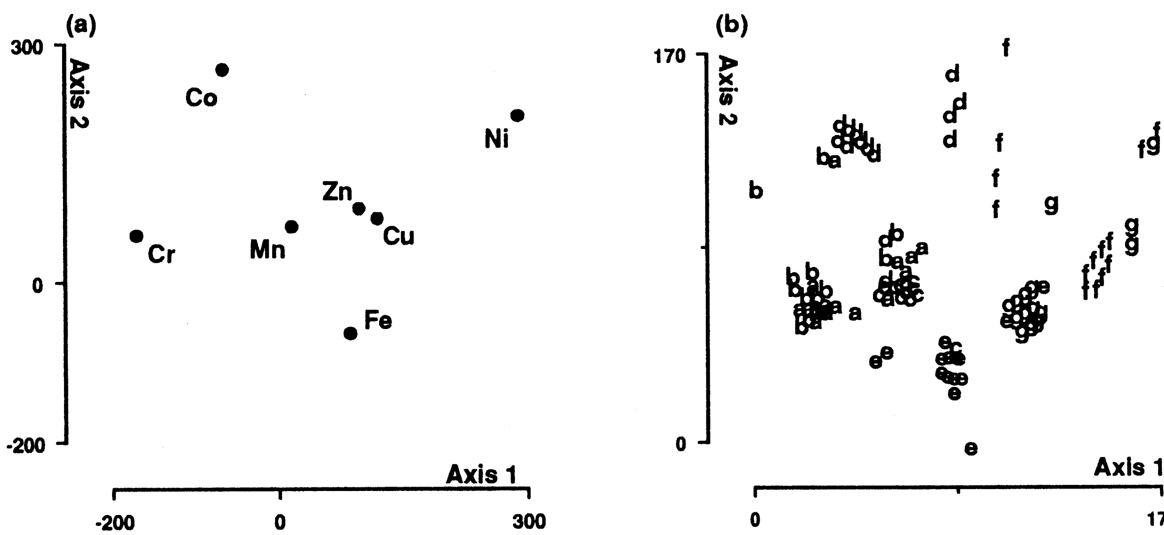

Fig. 4—A detrended correspondence analysis of Co, Cr, Cu, Fe, Mn, Ni and Zn levels in seven species of mollusc. Plots of axes 1 and 2 of (a) metals and (b) species. Key to species: a = *Cerastoderma edule*, b = *Mytilus edulis*, c = *Patella vulgata*, d = *Nucella lapillus*, e = *Monodonta lineata*, f = *Littorina littorea*, g = *Littorina obtusata*.

much higher, than the levels obtained by Wilson (1980). Winter levels of Zn and Mn in the Shannon Estuary were higher than the comparable values of Wilson (1980). The Shannon Estuary levels of Mn and Cr were higher than and the Cu levels were similar to the values at the east-coast sites (Wilson 1980).

In an experimental study of Ni levels in *C. edule*, Wilson (1983) found considerable variation between individuals and unusually high levels compared to other species. This was also evident in the present study, where the Ni levels had relatively large standard errors (see Fig. 1 and Table 1). Furthermore, some individuals had Ni levels of zero (below the detection limit of the atomic absorption spectrophotometer). Mean Ni levels were higher than in the other molluscs studied, with the exception of both *Littorina* species in winter only. Levels of Ni were higher in winter than in summer.

Mytilus edulis

In the present study, *M. edulis* (species b in Fig. 1) acted as a good indicator of Co, Mn, Cu, and Zn, although Bryan *et al.* (1980) considered *M. edulis* a moderate indicator of Co, a moderate to poor indicator of Cu, a good indicator of Cr, and a moderate indicator of Zn with high individual variability which, like *C. edule*, tended to regulate Zn. The results presented here (Fig. 2) show that *M. edulis* is a good all-round indicator of the metals studied. However, the season of sampling seems to be important for Cr, as low values occurred in *M. edulis* in summer although higher levels occurred in other species (*Nucella lapillus*, *Littorina obtusata*) at that time. Nevertheless, *M. edulis* is the most frequently used organism for biomonitoring levels of metals (see Goldberg *et al.* 1978) and the results presented here support this.

Patella vulgata

P. vulgata (species c in Fig. 1) showed a consistent accumulation of Cu at both sites and in both seasons. Bryan *et al.* (1985) considered *P. vulgata* a good indicator of Cu. In the present study *P. vulgata* also gave a consistent result for Mn and, to a lesser extent, Zn, and individual variability was low for both metals, as is evident from the low values of standard errors in Fig. 1. However, Bryan *et al.* (1985) considered *P. vulgata* a moderate indicator of Zn because of high individual variability in their study. Levels of all the metals in *P. vulgata* in winter were lower than Wilson's (1980) comparable east-coast values.

Nucella lapillus

At shore 1w, levels of Zn in *N. lapillus* were significantly different to those in all other species analysed (Table 1; Fig. 1, species d). When considered on a percentage scale (Fig. 2), the level of Cu in *N. lapillus* was higher than Fe. This suggests that *N. lapillus* may be a good accumulator of both Zn and Cu but there is high individual variability. Bryan *et al.* (1985) considered this species a moderate indicator of Zn but also stated that there was high individual variability. They also suggested that dissolved metals in *N. lapillus* resulted from their diet.

Shell colour of *N. lapillus* may depend to a large extent on diet (see Hyman 1967), with a dark shell resulting from a diet of *M. edulis* and a light shell from a diet of barnacles. At both shores in this study, *N. lapillus* exhibited light shell colour, suggesting a diet of barnacles. Rainbow *et al.* (1990) reported that barnacles were unable to excrete significant amounts of accumulated Zn and had a high Zn uptake. Consequently, the high levels of Zn in *N. lapillus* may come through the food-chain from barnacles. As filter-feeders, barnacles move large volumes of water across their cirri in order to feed; they feed mainly on plankton and suspended detritus, which are potentially high in metal levels. Thus some of the Zn levels may come from the suspended detritus in the water column via the barnacles. Rainbow *et al.* (1990) reported that the levels of Zn in barnacles were strongly influenced by season but that levels continued to accumulate throughout life. If this is so, the decrease in Zn levels in *N. lapillus* between winter and the following summer, as reported here, is not explained.

Monodonta lineata

There are no previous studies of metal bioaccumulation in *M. lineata*. Two sets of data are provided in this study (1w and 1s). *M. lineata* gave consistent results for Mn, and, to a lesser extent, for Zn, at the same site over two seasons (Fig. 1, species e). As in the case of *N. lapillus*, the Cu levels of *M. lineata* are comparatively high when examined on a percentage scale (Fig. 2).

The diet of *M. lineata*, a detrital sweeper, does not seem to be reflected in its capacity to accumulate metals, as detritus is metal-rich. Yet, with the exception of Cu, high levels of metals were not accumulated in this gastropod even though they are present in the immediate surroundings, as shown by the levels in the other species. It is known from studies by Catsiki *et al.* (1993) that accumulation of Cr by *M. turbinata* is influenced by pH levels and that Cu causes changes in tissue pH. These authors stated that *M. turbinata* accumulated more Cu when exposed only to Cu than when Cu and Cr were acting together. A similar situation may apply here in the case of *M. lineata*. The high Cu level in *M. lineata* may cause pH changes and this may affect the accumulation of Cr. It is unclear whether Cr has this effect on other metals.

With the possible exception of Mn, *M. lineata* is not an ideal indicator of metal contamination.

Littorina species

Much of the knowledge concerning *L. littoralis* may be based on either *L. obtusata* or *L. mariae* (see Hayward and Ryland 1995) and there are no comparative studies of metal levels in these two species. Some of the Zn levels in *L. obtusata* may be bioaccumulated from *Fucus vesiculosus*, as *L. obtusata* is frequently found grazing on this alga. *Littorina obtusata* had comparatively high levels of Mn but much lower levels of the other metals studied. Literature on the ability of *L. obtusata* to bioaccumulate metals, including Mn, is scarce and the subject merits further investigation.

Littorina littorea had higher levels of Co, Mn and Fe in summer than in winter. Bryan *et al.* (1985) considered *L. littorea* a poor indicator of Co. In the present study, it was not a good overall indicator of any of the metals studied because of high variability between sites. This conclusion is supported by a study of Co, Cr, Cu and Zn (Bryan *et al.* 1983) and a histochemical study involving Fe, Cu and Zn (Truchet *et al.* 1990). Neither study included Mn. However, the results presented here indicate high individual variation of Mn at site 2s. When examined on the percentage scale (Fig. 2), *L. obtusata* had relatively high values of Co.

Winter levels of Mn, Cr, Cu, Zn and Fe in this study were lower than the east-coast sites (Wilson 1980). Winter levels of Ni were much higher in the two species of *Littorina* than in Wilson's (1980) study. However, levels of Ni in both species were below the detection limits in summer. It is difficult to explain this seasonal difference, as Ni was present at the same sampling occasion in other species (*C. edule* and *M. edulis*). This implies that the season of sampling is of utmost importance when using *L. littorea* as an indicator species of metal contamination.

ALGAE

Seaweeds reflect the ambient dissolved levels of metals. Many studies (Cullinane and Whelan 1982; Bryan 1983; Bryan *et al.* 1985) have suggested that seaweeds are good indicators of heavy metal pollution, and several authors (e.g. Forsberg *et al.* 1988) have concluded that brown seaweeds are the best for biomonitoring purposes because of their wide distribution in estuaries. The results (Fig. 3) show that *Ulva lactuca* acted as a good indicator of Cr, Cu, Fe and Mn. High levels of Zn were found in *Pelvetia canaliculata* and *Fucus vesiculosus*. These results suggest that *P. canaliculata* is a moderate indicator of Zn owing to high individual variability, as is evident in Fig. 3. Otherwise, only low levels of metals occurred in algae in the present study.

MULTIVARIATE ORDINATION

There appear to be no previous studies using a multivariate approach to the analysis of these types of data. The detrended correspondence analysis ordination (Fig. 4) is interesting for a number of reasons. The ordination of the metals suggests that Cr, Co and Ni are acting independently whereas the other metals seem to form a cluster in the middle of the graph. The ordination of the species is interesting in the way that related species cluster together: the bivalves form a single cluster and the gastropods another; the two littorinids cluster together. Since the ordination is based only on metal levels, this implies that the related species have somewhat similar metal uptake and bioaccumulation mechanisms. The two *Littorina* species were shown above to have high Ni levels, and the multivariate ordination of metals and species also supports this conclusion. Of the gastropods, *Patella vulgata* clustered closest to the bivalves. It is interesting to speculate that the feeding of *P. vulgata* (scraping of algae from rock surfaces) is more closely related to the filter-feeding of bivalves than to the grazing or predatory diets of the other gastropods, possibly suggesting a dietary origin for the levels of Cu, Mn and Zn associated with this species.

OVERALL CONCLUSION

This comparative study of heavy metals in molluscs shows that significant differences exist between the results obtained for different species and between sites and seasons. Variations over time are partly due to changes in metabolic processes in molluscs during the year, e.g. seasonal changes, maturity and spawning. The results show that species-specific metal bioaccumulation occurs and that this is seasonally specific and site-specific in some species. Thus biomonitoring programmes must carefully evaluate the appropriate biomonitoring species, and sampling dates must be considered when comparing results from different studies.

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